GEOFOOD – RAS and heating installation in The Netherlands

Half-yearly update report no. 2 – November 2019

Alexander Boedijn[1], Eric Poot[1], Carlos Espinal[2] and Rob van de Ven[2]

1. Wageningen University & Research, 2. Landing Aquaculture

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Address

Wageningen University & Research, BU Greenhouse Horticulture
Violierenweg 1, 2665 MV Bleiswijk
P.O. Box 20, 2665 ZG Bleiswijk
The Netherlands
+31 (0) 317 - 48 56 06
glastuinbouw@wur.nl
www.wur@glastuinbouw.nl

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The RAS- and aquaponic research facilities for the GEOFOOD project, located in Bleiswijk, the Netherlands, have been completed early May 2019. The system has been successfully brought to full operation after addressing several start-up issues. This report summarizes the overall progress, changes made as well as lessons learned and suggested best practices.

1 Progress in the research facilities

1.1 Timeline

The timeline in figure 1 summarizes the key processes and milestones during the operation of the aquaponic research facilities. The main goal during the period May until November 2019 has been to stock the system with fish and achieve stable production conditions. Two failed attempts to stock pikeperch in the system were followed by a combined stocking of one batch of tilapia (successful) and one batch of pikeperch (unsuccessful). After the third failed pikeperch stocking attempt, it was decided to further stock the system with tilapia to achieve project objectives within the time available. A full tilapia production run would also allow to test the design limits of the system in short time, due to the fast-growing nature of the species. Batch 1 and 2 of tilapia consisted of 50 and 1500 fish entering the facility at 0.2 grams of weight. A third batch is planned for December 2019.

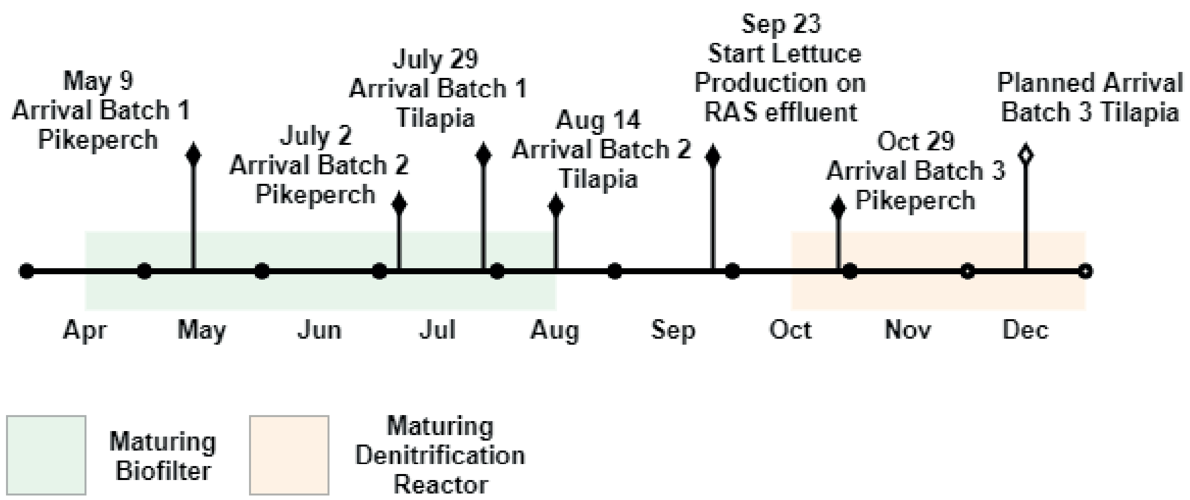


Figure 1 *Timeline summarizing the progress of the aquaponic research facilities in Bleiswijk, The Netherlands, during May – November 2019.*

The microbiome of the aquaponics system was matured initially by feeding the biofilter with ammonia. Later, with fish in the system, more bacterial communities would develop as fish sludge was loaded into the denitrification reactor and allowed to be anaerobically digested. Plant production started up towards the end of September when fish stocking density had sufficiently increased and the biofilter matured. This way trials could be set up to grow lettuce in separate systems containing RAS water and a standard hydroponic solution. The standard solution is water that has been disinfected using UV and then mixed as a nutrient solution for fertigation. The RAS effluent was analysed and corrected by adding missing nutrient compounds to reflect the same nutrient profile of the control. The goal of the trials is to build on previous work in which a similar experiment showed an increased performance of lettuce production on RAS effluent. A possible explanation would be the beneficial interaction between microorganisms and/or metabolites present in the RAS water and the plant roots, compared to the control water which is virtually devoid of micro-organisms (Goddek and Vermeulen 2018).

1.2 Switching from pikeperch to tilapia

One of the main changes has been the switch in cultivated fish species; from pikeperch (*Sander lucioperca*) to tilapia (*Oreochromis niloticus*). Pikeperch was selected for the GEOFOOD project because it is a high-value freshwater fish, posed as one of the candidates for EU aquaculture diversification. Though full efforts were made to set up a production cycle for pikeperch none of the batches that were introduced to the system subsisted. After three failed attempts of stocking the RAS, it was decided to switch to another fish species. The following species were considered:

- **Perch**: available from Asialor (France), and supposedly a bit more robust and easy to deal with than pikeperch. The fish grow a lot smaller (harvest size 200g), so a lot more fish is needed (5000 pcs per batch) to reach system capacity. However, for a potential business case, this might be an interesting species. Another advantage is that the existing pikeperch feed can be used.

- **Tilapia**: easy to acquire and a much more robust species. The fish is only available at a starting size of 0.2 grams, which means that considerable time is required to reach the maximum capacity of the system. Also, less interesting to consider for a business case.

- **Eel**: should be available locally, but the system will need to be modified to keep the eel from escaping tanks. The consortium partners have no expertise so consultancy from an external party would be needed.

- **Catfish**: should be available locally. They are a low value species but very robust. They take transport really well and grow fast, so the system could be pushed in terms of feed load and thus get a wider range of data. For a business case, it is a less interesting species.

- **Sturgeon**: should be available locally, but they are expensive fish to acquire. Also, density cannot be too high, and the feed load is limited, so the capacity of the system cannot be tested. It is an interesting species for a business case.

- **Carp**: availability within WUR, otherwise from German suppliers. They take a wide range of temperatures, are hardy and take high densities. However, commercially not interesting.

- **Omega perch**: we will most likely not be able to get fish, as there is only one supplier (in Belgium) who probably will not sell their fish. It would be an interesting fish to work with, also from a commercial point of view.

- **Salmon or trout**: are cold water species, and therefore not suitable for GEOFOOD.

After weighing advantages and disadvantages, it was decided to continue with tilapia. Tilapia is a more hardy species of which quality fingerlings are more readily available via Til-Aqua, a breeding company located in the Netherlands. Tilapia is a warm water species that is cultivated at similar temperatures as pikeperch, but grows at a faster rate. A fast-growing species was needed to complete the project objectives within the available time frame. General findings about pikeperch as a species for RAS production as well as insights regarding the loss of each batch are discussed in section 2 of this report.

1.3 Heating system

The heating system setup, as described in the half-yearly update report of May 2019, has been working properly. RAS system water temperature can be accurately controlled, and energy consumption is recorded. Figure 2 shows the daily mean temperature from the end of July until November 2019.
During this period water temperature was adjusted on several occasions to follow protocols for stocking and raising both pikeperch and tilapia. On the 28th of July system water temperature was set at 24°C to approximate the temperature at which the first batch of tilapia was stocked. The system had no fish at that point. After the arrival and acclimation of the tilapia, temperature was gradually increased to the species' optimum. After the second batch of tilapia had acclimated to the system, temperature was raised slightly from 27°C to about 28°C, as recommended by the breeding company and literature (Timmons, Guerdat et al. 2018).

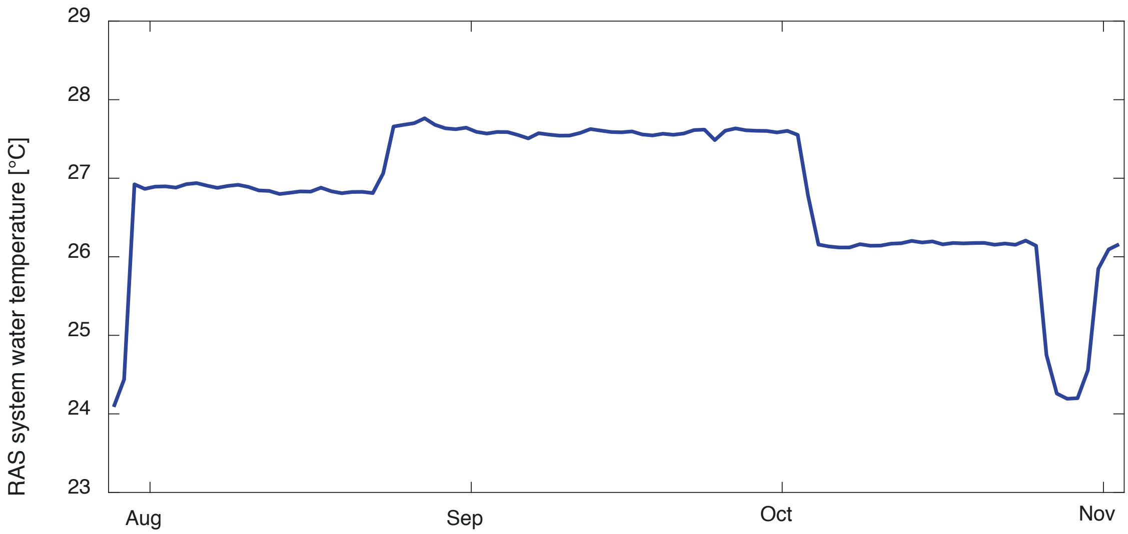


Figure 2 *Daily mean RAS system water temperature.*

Towards the end of September, it was decided to try once more to stock pikeperch. This would result in a polyculture system with both tilapia and pikeperch. Since 28°C was considered too high for pikeperch, the setpoint was changed to 26°C in the beginning of October to give the tilapia time to acclimatise.
Finally, expecting the arrival of the third batch of pikeperch, temperature was lowered again to approximate transport temperature to avoid shocking the incoming fish. Since this batch of pikeperch did not subsist, a third tilapia batch is planned for December 2019 to further stock the system and temperature will be gradually increased again to 28°C. Target water temperature is a key parameter that influences heat use, the temperature fluctuations as described above will therefore be accounted for when analysing the data and validating the GEOFOOD energy flow model.

To get a first indication of the model's performance a load duration curve was created based on the daily heat demand. Figure 3 shows both measured and simulated curves. Though the model overestimates heat demand, the results are promising and explainable. Some input parameters of the greenhouse still have to be fine-tuned based on further analysis such as the R-value as well as infiltration- and ventilation rates. Furthermore, the model enforces ventilation based on a relative humidity threshold which is not the case in the actual greenhouse. Finally, the actual weather data must be used as an input rather than values from a dataset containing yearly averages.

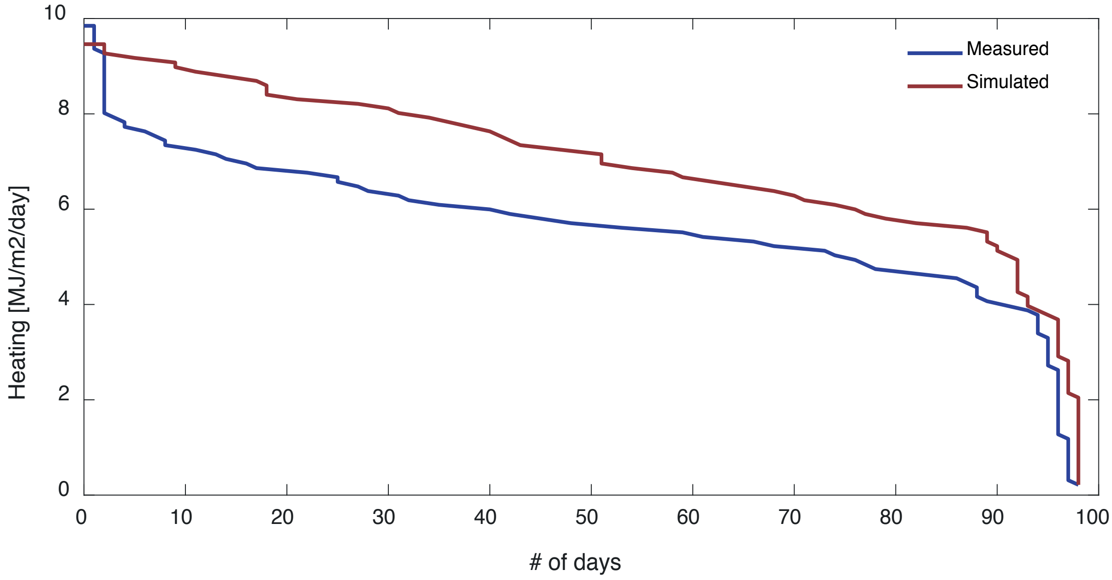


Figure 3 *Measured and simulated load duration curves of the RAS daily heat demand.*

2 Lessons learned and best practices

2.1 Evaluating water sources

Water quality is key for aquaculture and aquaponics and there are many parameters that need to be considered. An overview of the water quality parameters that are monitored and managed within the RAS and aquaponic research facilities is presented in table 1.

Table 1
Water quality parameters monitored and managed within the RAS and hydroponic research facilities.

Water quality parameter	Symbol	RAS / Hydroponics	Action
Electrical conductivity	EC	Hydroponics	Measured infrequently for plant production purposes
Nutrient content	-	RAS/ Hydroponics	Measured infrequently for plant production purposes
Acidity/Basicity	pH	RAS/ Hydroponics	Monitored continuously and managed using automatic dosing of buffer solution
Alkalinity/Hardness	$CaCO_3$	RAS	Monitored frequently and managed using automatic dosing of buffer solution
Ammonia/Ammonium or TAN	NH_3 / NH_4^+	RAS	Measured frequently and actions are taken accordingly (e.g. reduce feed load and initiate a water exchange)
Nitrite	NO_2^-	RAS	Measured frequently and actions are taken accordingly (e.g. reduce feed load and initiate a water exchange)
Nitrate	NO_3^-	RAS	Measured frequently and actions are taken accordingly (e.g. reduce feed load and initiate a water exchange)
Temperature	T	RAS	Monitored continuously and automated management
Oxygen	O_2	RAS/ Hydroponics	Monitored continuously and automated management
Heavy metals	-	RAS	Measured infrequently to assure quality standards

Water quality starts with the quality of the water source. At the start of the project rainwater, collected by the greenhouse roof and stored in an open basin, was selected as suitable water source. After UV and ozone treatment the water is free of pathogens and rainwater contains few minerals that require filtering.

Unfortunately, the rainwater did contain traces of aluminium (Al), copper (Cu) and zinc (Zn). Though the concentrations would not impact plant production, levels were slightly higher than thresholds found in literature for most fish species (Timmons, Guerdat et al. 2018). As the rainwater runs through aluminium gutters it most likely collects aluminium particles. Copper and zinc are not present in the greenhouse construction and must either be present in the rainwater itself or must be deposited in the basin due to nearby industrial activities or traffic.

To avoid build-up of heavy metals in the water, the main supply line was changed (23rd of October) to take in groundwater that is first filtered and then treated using reverse osmosis (RO). This water source contains almost no traces of heavy metals. The rainwater is still used as a back-up source. Appendix A contains an overview of the performed water quality analyses.

Best practice: Before construction, always perform a full water quality analysis, including heavy metals, for all available water sources. This will indicate the potential and limitations of each source. It is recommended that a back-up water source is accounted for within the design.

2.2 RAS within greenhouse construction

The RAS facility was constructed within a greenhouse compartment, part of a larger greenhouse research facility. An unforeseen negative effect was that greenhouses are almost never completely watertight. During heavy or long periods of rainfall, water leaked into the compartment and subsequently into the RAS system water. This is a hazard since pathogens and pollutants can enter the system as the water runs along:

- Greenhouse cladding (bird droppings, decaying organic material such as leaves, depositions of pollutants)
- Mechanical parts (lubricating oil)
- Screens (heavy metals and residues from pesticides of previous crop trials)

To address this influx of possibly contaminated water a carbon filter was installed that runs on a side-loop, thereby cleaning the system water. In addition the leaks were repaired.

Best practice: Before construction, locate big leaks and repair these as best as possible. Thoroughly clean the inside and outside of the greenhouse or building. If the facility has been used for other purposes, check if pesticides or other contaminants have been used and whether residues form a hazard.

A foreseen effect on system management was the control of indoor climate. A well-insulated building has a relatively simple climate control system. Greenhouses on the other hand need a detailed control strategy based on the local outdoor climate conditions. For instance, to avoid overheating during the summertime, a ventilation protocol was designed and implemented that allows for cooling during the night-time without compromising the light level thresholds (i.e. timed opening of windows and screens).

2.3 Pikeperch as a RAS species

Pikeperch was one of six species selected for the European project DIVERSIFY (FP7, GA 603121), a five-year project for the diversification of European aquaculture using new/emerging finfish species. The project was completed in November 2018 and a lot of progress was made to advance pikeperch as a species reared in RAS, including publications workshops and technical leaflets.

However, pikeperch production is still an emerging sector, which posed several challenges for the GEOFOOD project. First, acquiring a healthy batch of fish of appropriate and uniform size is not an easy task. There are not many suppliers nor are all of these long-established businesses that guarantee availability as well as quality. Second, from visits to several facilities that breed and grow pikeperch in RAS it became clear that protocols and opinions on good practice vary. This complicated the task of setting up and implementing rearing and handling protocols because not all staff were experienced fish culturists.

Furthermore, one of the major bottlenecks for further expansion of pikeperch culture that was identified within the DIVERSIFY project is the high sensitivity of pikeperch to any stressors, handling and husbandry practices, which leads to high and sudden mortalities. Unfortunately, this has also been the experience within the GEOFOOD project. The following batches of pikeperch were unsuccessful:

• **Batch 1**
Date: 9 May 2019
Size: 1100 fish of 157 grams
Cause of mortalities: Ramping up the feed input too fast combined with an immature and/or failing biofilter resulted in a spike in nitrite concentration up to 10 mg/L that lasted several hours to half a day before appropriate action could be taken. The entire batch was lost over a period of 4 days.

Best practice: Ensure biofilter functionality by testing conversion speed and capacity with ammonia and fish feed input. Gradually increase the feed input, especially when stocking larger fish. Monitor system nitrogen values (i.e. TAN, nitrite and nitrate) at a higher frequency (e.g. every 3-6 hours) after a batch enters the system. The same holds for other key water quality parameters.

• **Batch 2**
Date: 2 July 2019
Size: 520 fish of 250 grams
Cause of mortalities: Poor handling during transport in combination with a *Trichodina* outbreak resulted in the loss of the entire batch over a period of 24 hours. A clinical report by LandIng Aquaculture is available and attached to this report as Appendix B. The RAS was afterwards cleaned and treated using formaline to assure no *Trichodina* could survive until the next stocking.

Best practice: Since pikeperch is highly sensitive to stressors extra care must be taken during transport with respect to water quality parameters, handling and acclimation. Ensure quality of transport and handling together with suppliers. Request health certificates if possible. Request sampling of the fish for diseases or parasites upon arrival if possible. Use an acclimation tank separate from the RAS to receive the fish and thereby decrease the chance of contaminating the system.

• **Batch 3**
Date: 29 October 2019
Size: 1000 fish of 50 grams
Cause of mortalities: Probable cause was stress due to a longer period of transport. LandIng Aquaculture accompanied and supported the transport to ensure handling was done with care and oxygen levels were monitored. Acclimation went well and the fish did not show a high level of stress or injuries after arrival. Still, about 80% of all fish died within 12 hours after transport.

In the night of 30 October at about 3:00 the Oxyguard system did not record any data for a period of 30-45 min. However, before and after this gap water quality parameters, especially oxygen, were within acceptable range. If a temporary loss of power did occur, the magnetic emergency valves would have triggered oxygen dosing. Also, if a serious loss of oxygen did occur, it did not at all affect the batches of tilapia living in the RAS. There were no indications found of nitrite poisoning, suffocation or *Trichodina*.

Best practice: Pikeperch is very sensitive to transport. The species is prone to sustain injuries in higher densities due to their sharp teeth and dorsal fins. Especially their eyes are vulnerable. Transporting larger fish increases these risks. It is advised that not only oxygen but also pH, nitrogen compounds and alkalinity of the transport water are monitored and controlled. LandIng Aquaculture advises using a mild dose of aesthetic for long transports and/or large fish. Finally, future aquaculture and aquaponics research should be aware that pikeperch is a challenging species for inexperienced fish culturists and the safest option to stock a new system is to do it very gradually and only use small fish (<20 grams).

Literature

Goddek, S. and T. Vermeulen (2018).
"Comparison of Lactuca sativa growth performance in conventional and RAS-based hydroponic systems." Aquaculture International 26.

Timmons, M. B., T. Guerdat and V. B. J. (2018).
Table 2.4 Optimum Temperature Ranges (°C) for Representative Aquaculture Species. Recirculating Aquaculture. Ithaca, NY, Ithaca Publishing Company LLC.

Timmons, M. B., T. Guerdat and V. B. J. (2018).
Tables 2.2a and 2.2b Water Quality Criteria for Aquaculture. Recirculating Aquaculture. Ithaca, NY, Ithaca Publishing Company LLC.

Appendix 1 Water quality analyses for heavy metals

The inlet water and system water were tested for heavy metals. To avoid build-up of mainly aluminium, copper and zinc, the inlet water was changed to groundwater treated by a reverse osmosis installation (RO) instead of rainwater. Threshold values are based on Timmons, Guerdat et al. (2018)

		Samples 11-7-2019		**Samples 20-9-2019**		**Samples 27-10-2019**	
Element	**Threshold**	**Inlet water**	**System water**	**Inlet water**	**System water**	**Inlet water****	**System water**
	[ug/L]	[ug/L]	[ug/L]	[ug/L]	[ug/L]	[ug/L]	[ug/L]
Aluminium (Al)	10	6.6	5.9	12.1	12.4	1.2	4.5
Arsenic (As)	50	0.1	0.56	0.15	0.81	0.1	2.7
Barium (Ba)	5000	2.9	131	124	161	5.4	40.5
Cadmium (Cd)	0.25*	0.37	0.1	0.1	0.1	0.1	0.1
Chromium (Cr)	-	0.1	0.1	0.1	0.12	0.1	0.1
Cobalt (Co)	-	0.2	0.2	0.2	0.2	0.2	0.2
Copper (Cu)	9*	19.3	1.6	28.7	4.7	2.2	2.2
Mercury (Hg)	20	0.05	0.05	0.05	0.05	0.05	0.05
Lead (Pb)	20	2.6	0.1	2.1	0.1	0.24	0.1
Nickel (Ni)	100	4.9	0.21	4	0.4	0.1	0.3
Tin (Sn)	-	1	1	11.2	1	1	1
Zinc (Zn)	120*	200	10.1	130	16.4	3.2	6.7
Silver (Ag)	3	0.5	0.5	0.5	0.5	0.5	0.5

* Threshold assuming a hardness of 100 mg/L as $CaCO_3$

** Inlet water source was changed to RO treated groundwater on 23 October 2019

Appendix 2 Clinical case report GEOFOOD batch 2 Pikeperch

Clinical case report

GEOFOOD RAS
4th July 2019

LandIngAquaculture

Author: Carlos Alberto Espinal

Background

520 pikeperch (*Zander lucioperca*) of approximate weight 250 grams were delivered from Fish2Be to WUR greenhouse horticulture on the 3rd of July 2019 at 13:00.
Upon arrival, Fish2Be allowed for a short acclimation period of approximately 5 minutes before starting to net the fish out of the transport truck. There was an attempt by Fish2Be to net the fish into dry buckets without water. The WUR staff requested that the buckets were at least filled with water before receiving the fish.
Fish were hauled into the GEOFOOD recirculation system in approximately 20 minutes.
Water quality in the GEOFOOD system has remained stable for the week prior to the fish stocking, with values:

- Dissolved oxygen >7.5mg/l
- pH 7.3-7.8
- Temperature: 25-26°C
- Total ammonia nitrogen <0.5 mg/l
- Nitrite (NO_2) <0.5 mg/l
- Nitrate (NO_3) 100-130 mg/l
- Alkalinity: approx. 70mg/l $CaCO_3$
- Salinity: 2 ppt

Water quality records are available for consultation.

Case description

Upon stocking, most fish showed signs of stress such as dark coloration and lethargy. About 50% of the stock showed normal swimming behaviour. One mortality upon stocking was recorded.
On the 3rd of July at 2:00, a high-water level alarm on the stocked tank prompted the WUR staff to visit the system. The high-water alarm was triggered by accumulation of dead fish on the tank's outlets. Approximately 200 mortalities were recorded. LandIng Aquaculture and WUR were present at the system at 8:30 that day. Quick loss of the remaining stock was observed for the rest of the day. Due to the rapid loss of fish, no treatments were applied.

Behavioral changes in the stock through the day included – in approximate order:

- Dark coloration
- Lethargy
- Erratic swimming, dashing across the water column
- Increased breathing effort, often jumping out of the water or hanging from the water surface
- Increased lethargy, incapacity to maintain body coloration
- Loss of balance
- Death by apparent hypoxia, with flared opercula and extended gills

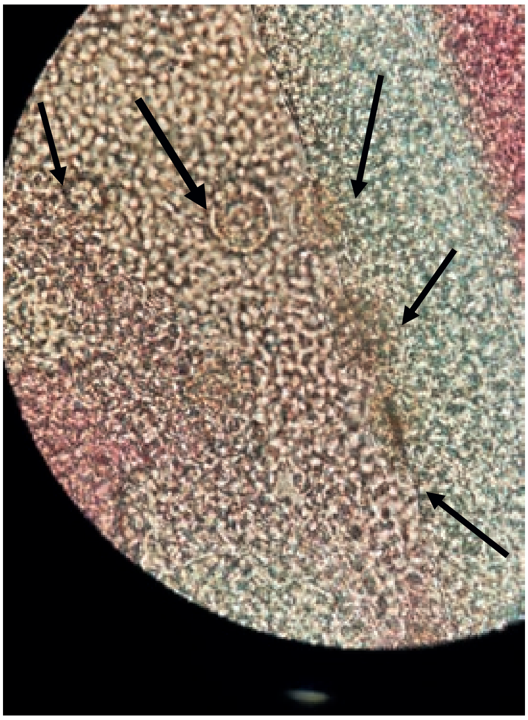
Gross examination showed clean bodies, some with small skin and fin damage from transport, some with eye damage from transport/netting. This prompted us to perform necropsies on fish to find any signs of disease.

Gross examination of 7 mortalities showed clean visceral packages, no congestion, no fluid accumulation and no haemorrhaging. Organs were not examined further. Skin scraps from the fish showed no signs of external parasites.

Three near-death fish were euthanized to have their gills examined on the microscope. Gill lamellae were placed in glass slides with a drop of water and covered. Two glass slides were placed under an optical microscope and examined at 10, 20, 40 and 100X magnification.

At 100X magnification, parasites were observed attached to the edges of gill lamellae. These were lenticular, round and showed a rotating motion which is typical of *Trichodinids*. We found 4-5 parasites per image at 100x maginification. “Low” levels of *Trichodina* infection are reported to show 1 parasite per image at 100x magnification (Noga, 2010). The investigation was stopped after observing the parasites in the slides.



Nitrite poisoning was ruled out because:

- NO_2 levels reported in the system were <0.5 mg/l. Salinity in the system was increased to 2 ppt prior to fish stocking.
- Gills and blood in fish were bright red. No brown gills or blood were observed.

Conclusion

We presumptively diagnosed a rapid *Trichodinid* outbreak based on the findings (disease pattern, behavioural changes in the fish, followed by necropsy findings). *Trichodina* is often a mild disease, but it can also cause heavy mortalities, sometimes with the action of secondary pathogens (Noga, 2010; Woo et al., 2002). No microbiology or virology samples were taken.

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To explore the potential of nature to improve the quality of life

Wageningen University & Research,
BU Greenhouse Horticulture
P.O. Box 20
2665 ZG Bleiswijk
Violierenweg 1
2665 MV Bleiswijk
The Netherlands
T +31 (0)317 48 56 06
www.wur.nl/glastuinbouw

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